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The Effect of Carbaryl (Sevin) on Reproduction of the Rat and the Gerbil

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The Effects of Carbaryl (Sevin) on Reproduction of the Rat and the Gerbil. COLLINS, THOMAS F. X., HANSEN, WALTER H., and KEELER, HELLYN V. (1971). *Toxicol. Appl. Pharmacol.* **19**, 202–216. The pesticide carbaryl (Sevin) was fed at dietary levels of 0, 2000, 5000, or 10,000 ppm to Osborne-Mendel rats for 3 generations. At 10,000 ppm, fertility was impaired and no litters were produced for the second mating of the second generation. Marked effects from birth to day 4 were manifested, as seen in the survival index. Dose-related decreases were observed in the averages of litter size, number of liveborn progeny, number of survivors to day 4, and number weaned.

In a comparative study, 3 generations of gerbils were fed carbaryl at dietary levels of 0, 2000, 4000, 6000, and 10,000 ppm. No litters were produced for the second mating of the third generation at 10,000 ppm. Gerbils appeared to be more sensitive to carbaryl than rats since significant decreases were found consistently at low dietary levels, but the effects were not so clearly dose-related. As in rats, the survival of liveborn to day 4 was markedly decreased.

Recent studies with carbaryl,[1] a broad-spectrum carbamate insecticide, have introduced some doubts as to its safe use at presently allowed tolerances. Marliac (1964), Marliac *et al.* (1965), Ghadiri and Greenwood (1966), and Khera (1966) reported congenital malformations in chicken and duck embryos when carbaryl was applied directly to the embryo or injected into the yolk sac. In a feeding study with laying hens and roosters, decreased hatchability of eggs and greater numbers of teratogenic effects in the young were definitely related to increased dose level (Ghadiri *et al.*, 1967). Teratogenesis was noted in guinea pigs, but not in hamsters or rabbits, after administration of high levels of carbaryl during organogenesis (Robens, 1969).

Smalley *et al.* (1969) reported that after feeding carbaryl up to 30 mg/kg/day to miniature sows during their entire pregnancies, there was no indication of any influence on reproduction. However, clinical signs of acute carbaryl poisoning were produced in swine and closely resembled those noted after other cholinesterase inhibitors. In the chronic study, effects on the neuromuscular apparatus were the predominant clinical signs observed, and three distinctive patterns of myodegeneration were noted.

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[1] Carbaryl (common name for 1-naphthyl-*N*-methylcarbamate) was obtained from Union Carbide Company, Pittsburgh, Pennsylvania.

In another mammalian study involving beagle dogs, carbaryl was fed throughout the gestation period at 5 levels from 3.125 to 50 mg/kg/day (Smalley *et al.*, 1968). Effects observed included dystocia (difficult births) due to atonic uterine musculature, an apparent contraceptive effect at the highest dose level, and a teratogenic effect observed at all the lowest dose levels.

In addition to the studies mentioned above, other studies on carbaryl (Food and Drug Administration, unpublished data) have shown toxicity and possible teratogenic effects. In an attempt to clarify the effects on reproduction in 2 species of mammals, a 3-generation reproduction study was initiated using rats and Mongolian gerbils.

MATERIALS AND METHODS

A 3-generation reproduction study was conducted as described by Fitzhugh (1968). Groups of 20 Osborne-Mendel littermated rats of approximate weaning age were fed diets ad libitum of Purina laboratory chow containing 0, 2000, 5000, and 10,000 ppm of carbaryl (technical grade, 99% purity). At 100 days of age the rats were mated, and a record of the fertility of each pair was kept. In a few instances the group of 20 pairs was reduced for causes other than toxicity of the compound: death of a mate or premature littering by a female with young dying of exposure after passing through the mesh on the cage bottom. At birth, the first litter, F_{1a}, was observed for the number of stillborn and liveborn young and for abnormalities. The litters were observed again on day 4, and counts were made of the number and condition of the living pups. When the pups in a litter exceeded 10 at day 4, the number was reduced to 10. At weaning, the rats were sacrificed. The parents, F_0, were remated, and the same observations were made on the second litter, F_{1b}. At weaning, these animals were not sacrificed in toto, but 20 littermate pairs at each dose level were selected to produce the next generation. The same procedure was followed for succeeding generations except that animals of the third generation were killed and necropsied.

The gerbil was used in this study because our laboratory was investigating new species for toxicity testing and response of a gerbil to a classic 3-generation reproduction study was not known. Mongolian gerbils (*Meriones unguiculatus*)[2] were fed diets containing 0, 2000, 4000, 6000, or 10,000 ppm of carbaryl. The 3-generation reproduction study was conducted in the same way as that of the rats, except that 40 gerbils were used as controls, 30 were used at each of the 2000, 4000, and 6000 ppm levels, and 18 were used at the 10,000 ppm level. At weaning, F_{3a} and F_{3b} gerbils from the 0 and the 6000 ppm levels were preserved for histopathologic study. The only exception to the mating procedure occurred in gerbils at the 10,000 ppm dose level. Since no males survived to weaning in the second mating of the second generation, 2 males from the first mating of the second generation were used to produce the third generation.

Fertility, viability, survival, and weaning indexes were calculated according to the description by Fitzhugh (1968); each index is fully described in the Results section.

RESULTS

The fertility index (number of litters cast/number of females exposed to mating) reflects female ability to produce young. In a few instances, the mother devoured her

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[2] Obtained from Tumblebrook Farm, Brant Lake, New York.

young before they could be counted, sexed, or weighed. These litters were counted in the fertility index since the animals were fertile, but were discarded in terms of the remaining indexes since no numbers were available for calculations. The 2-tailed chi-square test was used to determine significant differences between each dose level and the control for each mating in each generation.

As seen in Table 1, female rat ability to produce young was significantly decreased only at the 10,000 ppm level. No litters were produced for the second mating of the second generation. The same impairment of fertility was observed in the gerbils at the highest dose level; second litters were not produced in the third generation (Table 2). Significant decreases appeared in a few gerbil litters at the 3 intermediate dietary levels, but no clear correlation with dose was apparent.

TABLE 1

FERTILITY INDEXES OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)							
		0		2000		5000		10,000	
Generation	Mating	FI[a]	%[b]	FI[a]	%[b]	FI[a]	%[b]	FI[a]	%[b]
1	1	20/20	100	20/20	100	20/20	100	17/20	85
	2	18/20	90	16/20	80	16/20	80	11/16	69
2	1	18/20	90	19/20	95	18/20	90	3/7	43[c]
	2	17/18	94	17/19	89	17/18	94	0/3	0[c]
3	1	20/20	100	20/20	100	18/18	100	—	—
	2	18/19	95	20/20	100	18/18	100	—	—

[a] Fertility index (number of litters cast/number of females exposed to mating).
[b] Percent females pregnant.
[c] Significant at $P < 0.05$.

TABLE 2

FERTILITY INDEXES OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)									
		0		2000		4000		6000		10,000	
Generation	Mating	FI[a]	%[b]	FI[a]	%[b]	FI[a]	%[b]	FI[a]	%[b]	FI[a]	%[b]
1	1	38/40	95	25/30	83	23/30	77[c]	28/30	93	15/18	83
	2	34/38	89	21/25	84	18/23	78	22/28	79	9/14	64
2	1	39/40	98	29/30	97	28/30	93	27/30	90	3/5	60[c]
	2	39/39	100	27/29	93	26/28	93	25/27	93	1/3	33[d]
3	1	40/40	100	26/30	87[c]	28/30	93	21/22	95	1/2	50[c]
	2	39/40	98	21/26	81[c]	25/28	89	17/21	81[c]	0/2	0[d]

[a] Fertility index (number of litters cast/number of females exposed to mating).
[b] Percent females pregnant.
[c] Significant at $P < 0.05$.
[d] Significant at $P < 0.01$.

TABLE 3

AVERAGE LITTER SIZE OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)							
		0		2000		5000		10,000	
Generation	Mating	No.[a]	Av.[b]	No.[a]	Av.[b]	No.[a]	Av.[b]	No.[a]	Av.[b]
1	1	216/20	10.80	200/20	10.00	157/18	8.72[c]	116/20	5.80[d]
	2	183/19	9.63	168/20	8.40	141/20	7.05	72/16	4.50[d]
2	1	190/20	9.50	164/20	8.20	127/20	6.35[c]	14/7	2.00[d]
	2	175/18	9.72	151/19	7.95	133/18	7.39	0/3	0[d]
3	1	173/19	9.11	184/20	9.20	126/17	7.41[c]	—	—
	2	184/19	9.68	225/20	11.25	158/18	8.78	—	—
1, 2, 3[e]	1	579/59	9.81	548/60	9.13	410/55	7.45	130/27	4.81
	2	542/56	9.68	544/59	9.22	432/56	7.71	72/19	3.79

[a] Total number progeny/number females exposed to mating.
[b] Average litter size per female exposed to mating.
[c] Significant at $P < 0.05$.
[d] Significant at $P < 0.01$.
[e] Not analyzed for statistical significance.

TABLE 4

AVERAGE LITTER SIZE OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)									
		0		2000		4000		6000		10,000	
Generation	Mating	No.[a]	Av.[b]	No.[a]	Av.[b]	No.[a]	Av.[b]	No.[a]	Av.[b]	No.[a]	Av.[b]
1	1	196/40	4.90	128/30	4.27	119/30	3.97	135/30	4.50	72/18	4.00
	2	162/38	4.26	90/25	3.60	95/23	4.13	101/28	3.61	42/14	3.00
2	1	228/40	5.70	152/30	5.07	134/30	4.47[c]	135/30	4.50[c]	16/5	3.20[c]
	2	204/39	5.23	132/29	4.55	134/28	4.79	135/27	5.00	4/3	1.33[c]
3	1	227/40	5.68	133/30	4.43[d]	148/30	4.93	113/22	5.14	6/2	3.00
	2	211/40	5.27	107/26	4.12[d]	129/28	4.61	90/21	4.29	0/2	0[c]
1, 2, 3[e]	1	651/120	5.42	413/90	4.59	401/90	4.46	383/82	4.67	94/25	3.76
	2	577/117	4.93	329/80	4.11	358/79	4.53	326/76	4.29	46/19	2.42

[a] Total number progeny/number females exposed to mating.
[b] Average litter size per female exposed to mating.
[c] Significant at $P < 0.01$.
[d] Significant at $P < 0.05$.
[e] Not analyzed for statistical significance.

Average litter size per female exposed to mating in rats and gerbils is shown in Tables 3 and 4, respectively. Statistically significant differences between average litter size per female exposed to mating for each dose level compared to the control were determined by the 2-tailed *t* test, which was used for each mating in each generation. Two-tailed *t* tests were done in the same manner to obtain the data shown in Tables 5–10.

A definite dose-related effect on litter size was seen in rats at both 5000 and 10,000 ppm (Table 3). Significant decreases appeared in all first matings at 5000 ppm and in all matings at 10,000 ppm. A few slight, nonsignificant decreases appeared at the 2000 ppm level. Combined data for all generations, matings 1 and 2, showed a consistent dose-related decrease. Although the greatest effects in the gerbils were seen at the 10,000 ppm level, significant decreases were found in the third generation at the 2000 ppm level (Table 4). The combined results for matings 1 and 2, all generations, in the gerbil showed decreases in litter size at all dietary levels, in contrast to only the 2 highest levels for the rat; but the results are not as clearly dose-dependent in the gerbil as in the rat.

The average number of liveborn young per female exposed to mating for rats and gerbils, respectively, is shown in Tables 5 and 6. Significant decreases in the numbers of liveborn rats were found at the 5000 and 10,000 ppm dietary levels. At the 2000 ppm level, decreases occurred in 4 of the 6 matings, but the results are not significant because of the large variation in progeny between females within a group. When the data for matings 1 and 2 of all generations were combined, a definite correlation was found between dose and number of liveborn.

Dose-related decreases in viability were seen in the gerbils (Table 6) at the 6000 and 10,000 ppm levels. Significant decreases were seen also at 4000 ppm in the second and third generations and at 2000 ppm in the third generation. The consistent decreases which occurred in all generations, although nonsignificant, might indicate biological damage and might also indicate that the gerbil is more sensitive than the rat. Combined results for all generations, matings 1 and 2, showed decreases in viability at all dose levels, but dose-dependency is not apparent.

The viability index (number of liveborn/total number born) reflects the loss of young through stillbirths. Although the animals were not observed continually, they were observed at least once daily, hence the index reflects a time period from birth to a maximum of 24 hr of age. No statistical tests were made on this index, nor on any of the following indexes. As shown in Tables 5 and 6, slight decreases in the ratios were observable sporadically at intermediate dietary levels and at the highest level in both rats and gerbils. In the second and third generations of both species, an insufficient number of young was produced to provide a valid sample size.

The average number of progeny surviving to day 4 per female exposed is shown in Table 7 and 8 for rats and gerbils, respectively. Significant decreases in survival of rats occurred only at the 5000 and 10,000 ppm levels. The scattered decreases found at the 2000 ppm level are not significant because of the large variation in the average numbers of control animals. Combined results for all generations, matings 1 and 2, showed a clear dose relationship. In the gerbils, carbaryl had a more pronounced effect on progeny survival to day 4 than in the rats, for significant decreases were seen in almost all matings at all test levels, the greatest effects appearing at the highest level. A clear dose-relationship can be observed in 4 of the 6 matings. When the data are combined for all generations, a dose-relationship can be seen at the 2 highest levels in mating 1, and for all dose levels in mating 2.

The ratio of number alive at day 4/number born alive, or survival index, indicates the immediate postnatal effects on the survival of the young. Decreases in this index were found in both rats and gerbils at all dose levels of carbaryl, and there was an indication that the effect was dose-related (Tables 7 and 8), the 10,000 ppm level in both species of

TABLE 5

VIABILITY DATA OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)											
		0			2000			5000			10,000		
Generation	Mating	No.[a]	Av.[b]	VI[c,d]	No.[a]	Av.[b]	VI[c,d]	No.[a]	Av.[b]	VI[c,d]	No.[a]	Av.[b]	VI[c,d]
1	1	209/20	10.45	0.97	196/26	9.80	0.98	148/18	8.22[e]	0.94	97/20	4.85[f]	0.84
	2	175/19	9.21	0.96	151/20	7.55	0.90	138/20	6.90	0.98	64/16	4.00[f]	0.89
2	1	187/20	9.35	0.98	159/20	7.95	0.97	115/20	5.75[f]	0.91	12/7	1.71[f]	0.86
	2	175/18	9.72	1.00	146/19	7.68	0.97	118/18	6.56[e]	0.89	0/3	0[f]	—
3	1	164/19	8.63	0.95	179/20	8.95	0.97	116/17	6.82	0.92	—	—	—
	2	182/19	9.58	0.99	221/20	11.05	0.98	153/18	8.50	0.97	—	—	—
1, 2, 3[d]	1	560/59	9.49	0.97	534/60	8.90	0.97	379/55	6.89	0.92	109/27	4.04	0.84
	2	532/56	9.50	0.98	518/59	8.78	0.95	409/56	7.30	0.95	64/19	3.37	0.89

[a] Total number liveborn/number females exposed to mating.
[b] Average number liveborn per female exposed to mating.
[c] Viability index (number liveborn/total number born).
[d] Not analyzed for statistical significance.
[e] Significant at $P < 0.05$.
[f] Significant at $P < 0.01$.

TABLE 6

VIABILITY DATA OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)														
		0			2000			4000			6000			10,000		
Generation	Mating	No.^{a}	Av.^{b}	VI^{c,d}	No.^{a}	Av.^{b}	VI^{c,d}	No.^{a}	Av.^{b}	VI^{c,d}	No.^{a}	Av.^{b}	VI^{c,d}	No.^{a}	Av.^{b}	VI^{c,d}
1	1	187/40	4.67	0.95	124/30	4.13	0.97	110/30	3.67	0.92	114/30	3.80	0·84	55/18	3.06^{e}	0.76
	2	157/38	4.13	0.97	89/25	3.56	0.99	92/23	4.00	0.97	94/28	3.36	0.93	36/14	2.57	0.86
2	1	224/40	5.60	0.98	144/30	4.80	0.95	117/30	3.90^{f}	0.87	121/30	4.03^{f}	0.90	15/5	3.00^{f}	0.94
	2	199/39	5.10	0.98	123/29	4.24	0.93	126/28	4.50	0.94	123/27	4.56	0.91	4/3	1.33^{f}	1.00
3	1	224/40	5.60	0.99	126/30	4.20^{f}	0.95	138/30	4.60^{e}	0.93	101/22	4.59	0.89	6/2	3.00	1.00
	2	211/40	4.27	1.00	104/26	4.00^{e}	0.97	123/28	4.39	0.95	81/21	3.86^{f}	0.90	0/2	0^{f}	—
1, 2, 3^{d}	1	635/20	5.29	0.98	394/90	4.38	0.95	365/90	4.06	0.91	336/82	4.10	0.88	76/25	3.04	0.81
	2	567/117	4.85	0.98	316/80	3.95	0.96	341/79	4.32	0.95	298/76	3.92	0.91	40/19	2.11	0.87

[a] Total number liveborn/number females exposed to mating.
[b] Average number liveborn per female exposed to mating.
[c] Viability index (number liveborn/total number born).
[d] Not analyzed for statistical significance.
[e] Significant at $P < 0.05$.
[f] Significant at $P < 0.01$.

TABLE 7

SURVIVAL TO DAY 4 OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)											
		0			2000			5000			10,000		
Generation	Mating	No.[a]	Av.[b]	SI[c,d]	No.[a]	Av.[b]	SI[c,d]	No.[a]	Av.[b]	SI[c,d]	No.[a]	Av.[b]	SI[c,d]
1	1	180/20	9.00	0.86	185/20	9.25	0.94	91/18	5.06[e]	0.61	34/20	1.70[e]	0.35
	2	168/19	8.84	0.96	130/20	6.50	0.86	88/20	4.40[e]	0.64	18/16	1.12[e]	0.28
2	1	172/20	8.60	0.92	118/20	5.90	0.74	52/20	2.60[e]	0.45	6/7	0.86[e]	0.50
	2	161/18	8.94	0.92	122/19	6.42	0.84	54/18	3.00[e]	0.46	0/3	0[e]	—
3	1	144/19	7.58	0.88	162/20	8.10	0.91	85/17	5.00[f]	0.73	—	—	—
	2	171/19	9.00	0.94	206/20	10.30	0.93	119/18	6.61	0.78	—	—	—
1, 2, 3[d]	1	496/59	8.41	0.89	465/60	7.75	0.87	228/55	4.15	0.60	40/27	1.48	0.37
	2	500/56	8.93	0.94	458/59	7.76	0.88	261/56	4.66	0.64	18/19	0.95	0.28

[a] Total number survivors to day 4/number females exposed to mating.
[b] Average number survivors to day 4 per female exposed to mating.
[c] Survival index (number survivors to day 4/number liveborn).
[d] Not analyzed for statistical significance.
[e] Significant at $P < 0.01$.
[f] Significant at $P < 0.05$.

TABLE 8

SURVIVAL TO DAY 4 OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)														
		0			2000			4000			6000			10,000		
Generation	Mating	No.^{a}	Av.^{b}	SI^{c,d}	No.^{a}	Av.^{b}	SI^{c,d}	No.^{a}	Av.^{b}	SI^{c,d}	No.^{a}	Av.^{b}	SI^{c,d}	No.^{a}	Av.^{b}	SI^{c,d}
1	1	142/40	3.55	0.76	71/30	2.37	0.57	66/30	2.20^e	0.60	67/30	2.23^e	0.59	17/18	0.94^f	0.31
	2	126/38	3.32	0.80	67/25	2.68	0.75	59/23	2.57	0.64	54/28	1.93^e	0.57	14/14	1.00^f	0.39
2	1	188/40	4.70	0.84	107/30	3.57^e	0.74	69/30	2.30^f	0.59	59/30	1.97^f	0.49	7/5	1.40^f	0.47
	2	181/39	4.64	0.91	96/29	3.31^e	0.78	69/28	2.46^f	0.55	62/27	2.30^f	0.50	2/3	0.67^f	0.50
3	1	181/40	4.52	0.81	91/30	3.03^f	0.72	70/30	2.33^f	0.51	62/22	2.82^f	0.61	3/2	1.50^e	0.50
	2	179/40	4.47	0.85	81/26	3.12^e	0.78	70/28	2.50^f	0·57	38/21	1.81^f	0.47	0/2	0^f	—
1, 2, 3^d	1	511/120	4.26	0.80	296/90	2.99	0.68	205/90	2.28	0.56	188/82	2.29	0.56	27/25	1.08	0.36
	2	486/117	4.15	0.86	244/80	3.05	0.77	198/79	2.51	0.58	154/76	2.03	0.52	16/19	0.84	0 40

[a] Total number survivors to day 4/number females exposed to mating.
[b] Average number survivors to day 4 per female exposed to mating.
[c] Survival index (number survivors to day 4/number liveborn).
[d] Not analyzed for statistical significance.
[e] Significant at $P < 0.05$.
[f] Significant at $P < 0.01$.

TABLE 9

WEANING DATA OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)											
		0			2000			5000			10,000		
Generation	Mating	No.[a]	Av.[b]	WI[c,d]	No.[a]	Av.[b]	WI[c,d]	No.[a]	Av.[b]	WI[c,d]	No.[a]	Av.[b]	WI[c,d]
1	1	154/20	7.70	0.86	176/20	8.80	0.95	83/18	4.61[e]	0.91	25/20	1.25[f]	0.74
	2	151/19	7.95	0.90	113/20	5.65	0.87	56/20	2.80[f]	0.67	12/16	0.75[f]	0.67
2	1	166/20	8.30	0.97	115/20	5.75	0.97	50/20	2.50[f]	0.96	5/7	0.71[f]	0·83
	2	154/18	8.56	0.96	110/19	5.78	0.90	49/18	2.72[f]	0.91	0/3	0[f]	—
3	1	141/19	7.42	0.98	154/20	7.70	0.95	84/17	4.94	0.99	—	—	—
	2	169/19	8.89	0.99	196/20	9.78	0.95	96/18	5.33[f]	0.81	—	—	—
1, 2, 3[d]	1	461/59	7.81	0.93	445/60	7.42	0.96	217/55	3.94	0.95	30/27	1.11	0.75
	2	474/56	8.47	0.95	419/59	7.11	0.92	201/56	3.60	0.77	12/19	0.63	0.67

[a] Total adjusted number survivors to day 21/number females exposed to mating. Adjusted number day 21 survivors = (number alive at day 21/number kept at day 4) × number alive at day 4.

[b] Average number survivors to day 21 per female exposed to mating.

[c] Weaning index (adjusted number survivors to day 21/number alive at day 4).

[d] Not analyzed for statistical significance.

[e] Significant at $P < 0.05$.

[f] Significant at $P < 0.01$.

TABLE 10

WEANING DATA OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)														
		0			2000			4000			6000			10,000		
Generation	Mating	No.^{a}	Av.^{b}	WI^{c,d}	No.^{a}	Av.^{b}	WI^{c,d}	No.^{a}	Av.^{b}	WI^{c,d}	No.^{a}	Av.^{b}	WI^{c,d}	No.^{a}	Av.^{b}	WI^{c,d}
1	1	132/40	3.30	0.93	70/30	2.33	0.99	62/30	2.07	0.94	63/30	2.10	0.94	10/18	0.56^e	0.59
	2	120/38	3.16	0.95	62/25	2.48	0.93	56/23	2.43	0.95	52/28	1.86	0.96	11/14	0.79^e	0.79
2	1	167/40	4.17	0.89	103/30	3.43	0.96	65/30	2.17^e	0.94	48/30	1.60^e	0.81	6/5	1.20^e	0.86
	2	166/39	4.26	0.92	92/29	3.17	0.96	61/28	2.18^e	0.88	58/27	2.15^e	0.94	2/3	0.67^e	1.00
3	1	161/40	4.02	0.89	82/30	2.73^e	0.90	62/30	2.07^e	0.89	50/22	2.27^e	0.81	3/2	1.50	1.00
	2	158/40	3.95	0.88	72/76	2.77^f	0.89	66/28	2.36^e	0.94	37/21	1.76^e	0.97	0/2	0^e	—
1, 2, 3^d	1	460/120	3.83	0.90	255/90	2.83	0.95	189/90	2.10	0.92	161/82	1.96	0.86	19/25	0.76	0.70
	2	444/117	3.79	0.91	226/80	2.82	0.93	183/79	2.32	0.92	147/76	1.93	0.95	13/19	0.68	0.81

[a] Total number survivors to day 21/number females exposed to mating.

[b] Average number survivors to day 21 per female exposed to mating.

[c] Weaning index (number alive to day 21/number alive to day 4).

[d] Not analyzed for statistical significance.

[e] Significant at $P < 0.01$.

[f] Significant at $P < 0.05$.

animals showing the greatest effect. The number of animals produced in the second and third generations at 10,000 ppm was insufficient to obtain a valid sample.

The average number of progeny weaned at day 21 per female exposed to mating is shown in Table 9 and 10 for rats and gerbils, respectively. In the rats, all litters of more than 10 were reduced to that number at day 4. Hence, the number of rat survivors at day 21 has been adjusted by multiplying the number alive at day 4 by the ratio of number alive at day 21/number kept at day 4. The litter size in the gerbils never exceeded 10.

In rats (Table 9), the largest decreases in weanings were at the 2 highest test levels, but there were slight decreases in 3 of 6 matings at the 2000 ppm level. Combined data for matings 1 and 2, all generations, showed a clearly dose-related decrease. Significant decreases in weanings were present at all dose levels in gerbils (Table 10) and 4 of 6 matings showed a clear dose-related effect. This is also borne out in the combined results.

Tables 9 and 10 also show the weaning index (adjusted number of survivors on day 21/number alive at day 4). The number of rat survivors was adjusted to reflect the animals culled at day 4. The weaning index is a measure of pup ability to survive to weaning from day 4 and is an indirect indication of the quality and quantity of the maternal milk. In rats, effects were seen in all generations at the 5000 ppm level and the two generations available at the 10,000 ppm level. Appreciable decreases in weaning index in gerbils were found at the 10,000 ppm level in the first generation, and sporadic decreases appeared at the 6000 ppm level.

TABLE 11

WEANLING WEIGHTS (IN GRAMS) BY SEX OF RATS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)							
		0		2000		5000		10,000	
Generation	Mating	M	F	M	F	M	F	M	F
1	1	46.0	43.7	36.2[a]	37.2[b]	26.7[a]	28.6[a]	22.2[a]	18.2[a]
	2	43.8	41.8	36.2[b]	33.8[a]	28.0[a]	28.3[a]	35.8[a]	21.2[a]
2	1	42.5	40.5	35.6[b]	34.5[b]	27.6[a]	25.2[a]	22.0[a]	18.0[a]
	2	45.0	42.5	34.2[a]	31.6[a]	24.2[a]	23.8[a]	—	—
3	1	46.0	43.9	35.9[a]	34.2[a]	26.2[a]	25.8[a]	—	—
	2	44.5	40.5	33.5[a]	31.3[a]	22.0[a]	21.9[a]	—	—

[a] Significant at $P < 0.01$.
[b] Significant at $P < 0.05$.

With a single exception, a dose-related decrease was observed in the weanling weights of rats after carbaryl treatment, as shown in Table 11. All rat weight decreases at all experimental dose levels were statistically significant. Weanling weights of gerbils, always lower than those of rats, were not significantly decreased at 2000 ppm (Table 12). At the intermediate levels, 3 significant decreases were seen at 4000 ppm, and 2 were seen at 6000 ppm. At 10,000 ppm, 6 of 9 weights were significantly decreased. The weight decreases in gerbils did not appear dose-related. Weanling weights of rats and gerbils were analyzed for statistical significance by the 2-tailed *t* test.

TABLE 12

WEANLING WEIGHTS (IN GRAMS) BY SEX OF GERBILS FED CARBARYL FOR 3 GENERATIONS

		Dietary level (ppm)									
		0		2000		4000		6000		10,000	
Generation	Mating	M	F	M	F	M	F	M	F	M	F
1	1	15.1	14.5	15.4	14.2	11.1[a]	13.3	13.1[a]	13.1	13.2[b]	13.8
	2	14.5	14.4	15.6	14.8	14.0	13.4	13.6	13.2	13.4	13.4
2	1	14.1	13.9	13.6	13.4	13.6	12.9	13.0	12.8	11.8[b]	9.5[b]
	2	13.9	13.8	13.3	13.0	14.0	14.3	13.3	13.7	—[c]	11.0[b]
3	1	14.2	13.3	13.4	12.9	12.9[b]	12.7	13.3	12.4	11.5[b]	12.0[b]
	2	14.4	14.6	14.4	13.3	13.4	12.4[b]	12.9	12.5[a]	—	—

[a] Significant at $P < 0.05$.

[b] Significant at $P < 0.01$.

[c] Since no male survived to weaning, 2 males from the first mating of the 2nd generation were used to produce the 3rd generation.

No grossly visible abnormalities were observed in any of the offspring produced in this study. When gerbil young from third generation control and 6000 ppm levels were studied histopathologically, no abnormalities were seen. Microscopic pathology was not done in rats.

DISCUSSION

Several advantages in the use of gerbils for 3-generation reproduction studies were noted and are listed here. With ease, they can be kept under laboratory conditions of temperature, humidity, and light similar to those for rats. They thrive on rat chow in pellet or ground form. If fed rat chow, they require water but no other additive to their diet. They have a high fertility rate and a short gestation period.

In addition to the 3 points above, this study suggests that gerbils are more sensitive to certain chemical compounds than are rats. Gerbils were more sensitive to the administration of carbaryl than rats, since at the 2000 ppm level, gerbils showed significant decreases in average litter size, in the average number of liveborn per female mated, average number of survivors to day 4, and average number of progeny weaned. Although decreases in these ratios were found in rats fed 2000 ppm, they were significant only at the 2 highest levels.

Further comparison of the results of rats and gerbils showed that the carbaryl-related effects in rats were more often correlated with dose level than in gerbils which showed a more randomized pattern of decrease.

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10,000	
M	F
13.2^b	13.8
13.4	13.4
11.8^b	9.5^b
—^{c}	11.0^b
11.5^b	12.0^b
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Previous studies on rats showed that 2000 ppm produced no effect on growth, while 10,000 ppm depressed growth in test rats and reduced the weight gain of mothers and nursing pups (Food and Drug Administration, unpublished data). The rats in this experiment showed growth depression at the high level, but not at the 2 lower levels. Hence, it is possible that some of the effects on rat reproduction at the 10,000 ppm level might be relatable to caloric deprivation. The weights of the gerbils in this study showed a dose-related decrease, and some of the effects on reproduction in gerbils might be due to the toxicity of the pesticide, especially at 10,000 ppm. The dose-related response in weight also suggests a greater sensitivity of the gerbil to carbaryl.

Each of the 4 indexes, fertility, viability, survival, and weaning, reflects the effects of the compound on a particular developmental span of time. At 10,000 ppm, carbaryl showed a definite trend toward the production of sterility in both rats and gerbils. In addition to progressive sterility, the effects of carbaryl were most apparent in the period from birth to day 4, as reflected in the low values of the survival index.

Orlova and Zhalbe (1968) reported reduced rat fertility which was correlated with decreased sperm motility and changes in the enzymatic activity of both testes and ovaries. Spermatogenic damage and changes in the duration of the phases of the estrous cycle in rats due to carbaryl were similarly reported by Vashakidze *et al.* (1966, 1967). Any similar damage to rat sperm and ovaries could have resulted in the reduced fertility indexes observed in the present study. Previous studies were not available for comparing the present gerbil results, but the decreased fertility indexes at 10,000 ppm may indicate damage similar to that in rats. It has also been suggested that carbaryl exerts only an indirect influence on the reproductive organs by interfering directly with the hypothalamo-hypophyseal control functions (Shternberg and Rybakova, 1968).

The decreased survival indexes noted in both animal species from birth to day 4 are more difficult to correlate with specific studies. At only a few days of age, the animals appear to be more susceptible to metabolic damage resulting from carbarbyl administration than at an older age.

Adverse effects on reproduction were found at all dose levels of carbaryl but were most pronounced at the 2 highest test levels. A clear indication of a decrease in reproductive fitness was evident in both animal species at 2000 ppm, although at this dose level the decreases were significant only in the gerbil. We therefore estimate the no-effect level, based on 3-generation reproduction studies in rats and gerbils, to be below 2000 ppm.

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